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# Conservation Agriculture practices can reduce yield-scaled N<sub>2</sub>O emissions and the global warming potential of rainfed semi-arid agriculture

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## Abstract

Conservation tillage and crop rotations can potentially contribute to beneficial effects on soil quality. However, the impact of these practices on greenhouse gas (GHG) emissions and crop yields is not well defined, particularly in dry climates. A rainfed 2-year field-experiment was conducted to evaluate the effect of three long-term (17-18 years) tillage systems (Conventional Tillage (CT), Minimum Tillage (MT) and No Tillage (NT)) and two cropping systems (rotational wheat (*Triticum aestivum* L.) preceded by fallow, and monoculture wheat), on nitrous oxide, (N<sub>2</sub>O) and methane, (CH<sub>4</sub>) emissions, during two field campaigns. Soil mineral N, water-filled pore space, dissolved organic C, and grain yield were measured and yield-scaled N<sub>2</sub>O emissions, N surplus and Global Warming Potentials (GWP) were calculated. No tillage only decreased cumulative N<sub>2</sub>O losses (as opposed to MT/CT) during campaign 1 (the driest campaign with least synthetic N input), while tillage did not affect CH<sub>4</sub> oxidation. The GWP demonstrated that the enhancement of C sequestration under NT caused this tillage management to decrease overall CO<sub>2</sub> equivalent emissions. Wheat in

monoculture was associated with increased N<sub>2</sub>O fluxes during campaign 2 (normal year and conventional N input) and decreased CH<sub>4</sub> uptake, as opposed to rotational wheat. Conversely, wheat in monoculture tended to increase C sequestration and therefore to result in a lower GWP, but differences were not statistically significant. Grain yields were strongly influenced by climatic variability. The NT and CT treatments yielded most during the dry and the normal campaign, and the yield-scaled N<sub>2</sub>O emissions followed the same tendency. Minimum tillage was not an interesting tillage management considering the balance between GWP and yield-scaled N<sub>2</sub>O emissions (which were increased in a 64% compared with that of NT). Regarding the crop effect, wheat in rotation resulted in a 32% increase in grain yield and 31% mitigation of yield-scaled N<sub>2</sub>O emissions. Low cumulative N<sub>2</sub>O fluxes (< 250 g N<sub>2</sub>O-N ha<sup>-1</sup> campaign<sup>-1</sup>) highlighted the relevance of C sequestration and CO<sub>2</sub> emissions from inputs and operations in rainfed semi-arid cropping systems. This study suggests that NT and crop rotation can be recommended as good agricultural practices in order to establish an optimal balance between GHGs fluxes, GWP, yield-scaled N<sub>2</sub>O emissions and N surpluses.

**Keywords:** N<sub>2</sub>O emission, CH<sub>4</sub> emission, C sequestration, rotation, winter wheat, tillage

### **Highlights**

Different tillage treatments and wheat in rotation versus monoculture were evaluated in a long-term experiment.

No tillage and wheat in rotation resulted in similar or lower N<sub>2</sub>O emissions than conventional management.

Wheat in rotation (preceded by fallow) increased CH<sub>4</sub> uptake when compared with wheat monoculture.

Wheat in rotation increased grain yield and reduced yield-scaled N<sub>2</sub>O emissions.

No tillage decreased the net global warming potential due to enhanced C sequestration.

## **1. Introduction**

Agriculture contributes to 10-12% of the total global anthropogenic greenhouse gases (GHGs) (Stocker et al., 2013), through the release of nitrous oxide (N<sub>2</sub>O), carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>). The global warming potential (GWP), which is a concept that integrates the radiative properties of all GHG, expressed as CO<sub>2</sub> equivalents (CO<sub>2</sub>-eq), is very dependent on N<sub>2</sub>O emissions from agricultural crop systems. This gas, which is a by-product of microbial processes of nitrification and denitrification, (Firestone and Davidson, 1989), is released from soils after nitrogen (N) application (through fertilizers or crop residues). By contrast, in aerated soils CH<sub>4</sub> uptake normally reduces GWP, because the amount of CH<sub>4</sub> oxidized by methanotrophic microorganisms is normally higher than the amount produced by methanogenic microorganisms (Chan and Parkin 2001). Additionally, agricultural practices that favour carbon (C) sequestration (Robertson et al., 2000) are also considered as valuable strategies to reduce the negative effect of GHG emissions associated with crop production. Therefore, agricultural management practices (e.g. tillage, fertilization and crop rotation) must integrate the reduction of soil GHG emissions and the increase of C sequestration, while maintaining or enhancing crop yields to satisfy increasing global food demand.

Conservation agriculture, which involves crop rotations and reduced tillage (no tillage (NT) or minimum tillage (MT)), is currently common in Mediterranean climates

due to its effects on preserving soil fertility and increasing soil C sink (Kassam et al., 2012). These tillage practices often contribute to improve important abiotic parameters involved in the production and consumption of GHG from soils such as soil water content, aeration and soil organic C (SOC) (Martín-Lammerding et al., 2011; Plaza-Bonilla et al. 2014) compared to conventional tillage (CT). However, contradictory results on N<sub>2</sub>O and CH<sub>4</sub> fluxes have been reported (i.e. Pelster et al., 2011; Dendooven et al., 2012; Ball et al., 1999; Yonemura et al., 2014) due to interaction of tillage with several factors, e.g. soil type, climatic conditions (which determine the prevalence of nitrification or denitrification), nitrogen (N) fertilization rate, crop residues (type and management), and experiment duration (van Kessel et al., 2013).

The effect of crop rotations on GHG emissions is variable depending on rainfed/irrigated conditions, composition and management of previous crop residues (Malhi and Lemke, 2007), and mineral N remaining in soil from previous cropping phases. Cereal residues (high C:N ratio) can promote soil N immobilization when they are applied without an additional source of mineral N, consequently leading to a temporary reduction of N<sub>2</sub>O fluxes (Huang et al., 2004). However, other authors (Sarkodie-Addo et al., 2003) have observed an enhancement of denitrification losses when a mineral source is added together with high C:N ratio residues, providing an energy supply for denitrifying microorganisms. Addition of N fertiliser may also inhibit CH<sub>4</sub> uptake due to interference of enzyme activity responsible for CH<sub>4</sub> oxidation (CH<sub>4</sub> monooxygenase) with NH<sub>3</sub> monooxygenase (Dunfield and Knowles, 1995), depending on N rate (Aronson and Helliker, 2010). Different quantities of crop residue inputs are added to the soil under rotational wheat and monoculture wheat systems, which can affect net N<sub>2</sub>O and CH<sub>4</sub> production due to changes in soil C and N availability.

The influence of tillage and crop rotation on C sequestration has been previously assessed, showing promising but contrasting results depending on management (e.g. type and duration of rotation) and experimental (e.g. depth, number of years since the beginning of the experiment) factors (Baker et al., 2007; Álvaro-Fuentes et al., 2014; Triberti et al., 2016). Thus, to identify whether conservation tillage practices (MT/NT and crop rotation) can mitigate both soil GHG emissions and net GWP is still unclear, particularly in semi-arid areas where the weight of direct N<sub>2</sub>O losses is expected to be lower.

In rainfed semi-arid cropping systems, characterized by a high variability in total amount and distribution of rainfall, low N input systems are being promoted in order to match N input to the expected N uptake by crops (Kimani et al., 2003; Tellez-Rio et al., 2015), which may reduce N surplus and also N losses (van Groenigen et al. 2010). Therefore, combining Conservation Agriculture practices with adjusted N-input is expected to provide an optimum balance between GWP and crop yields in semi-arid agro-ecosystems. In this context, the main objective of this study was to evaluate the effect of three long-term tillage systems (CT, MT and NT) and two cropping systems (wheat in monoculture and wheat in a 4-year rotation with fallow as preceding crop) on N<sub>2</sub>O and CH<sub>4</sub> emissions over two campaigns. Additionally, crop yield, yield-scaled N<sub>2</sub>O losses (YSNE) and GWP were evaluated. We hypothesized that: 1) considering climatic conditions of this experiment and the low N input, low N<sub>2</sub>O emissions would be expected in all treatments; 2) emissions of N<sub>2</sub>O and CH<sub>4</sub> in monoculture winter wheat could be higher than in the rotational winter wheat, because of a combined effect of previous crop residues and N fertilizer application; and 3) NT would reduce net GWP as a result of the reduction of CO<sub>2</sub>-eq emissions from farm operations and the increase of C sequestration (Aguilera et al., 2013a).

## 2. Materials and methods

### 2.1. Site characteristics

A two-year study was carried out at “La Canaleja” Field Station (40° 32'N, 3° 20'W, 600 m), in Alcalá de Henares (Madrid, Spain), where a long-term tillage experiment began in 1994. Tillage systems and crop rotations including legumes and fallow have been assessed from that date. The soil was a sandy-loam *Calcic Haploxeralf* (Soil Survey Staff, 2010). The main physicochemical properties of the top soil layer (0-15 cm) were: sand, 50.8%; silt, 37.7%; clay, 11.5%; CaCO<sub>3</sub>, 41.6 g kg<sup>-1</sup>; pH<sub>H2O</sub>, 7.9 and EC, 121.3 µS cm<sup>-1</sup>. The site has a semiarid Mediterranean climate with dry summer. The 1994-2013 mean annual temperature and rainfall for this area were 13.5 °C and 402.7 mm, respectively.

Hourly rainfall and air temperature data were obtained from a meteorological station located at the field site. Soil temperature was measured in each tillage system by inserting a temperature probe 15 cm into the soil. Mean hourly temperature data were stored on a data logger.

### 2.2. Experimental design and management

The experiment was conducted from October 2011 to October 2013. The experimental design was a three-replicated split plot, divided into three main plots assigned to the three tillage systems (NT, MT and CT) in a randomized complete block design (Guardia et al., 2016). Each of the main plot was further divided into five subplots (10 x 25 m) assigned in completely randomized design to the phases of an annual crop rotation, involving fallow-wheat (*Triticum aestivum* L. var. Marius)–vetch (*Vicia sativa* L. var. Senda)-barley (*Hordeum vulgare* L. var. Kika), and also wheat in monoculture. In this study, we evaluated the effect of the three tillage systems

mentioned above (tillage factor) and two cropping systems (cropping factor): wheat in rotation and wheat in monoculture; during two campaigns with different climatic (i.e. rainfall amount) and management conditions (i.e. rate of N fertilizer at dressing) (campaign factor): 2011/12 (campaign 1) and 2012/13 (campaign 2), resulting in eighteen subplots (3 plots x 2 subplots x 3 replicates -blocks-).

Moldboard (20 cm depth) and chisel ploughs (15 cm depth) were used in autumn (early-November 2011 and late-October 2012, for campaign 1 and 2, respectively) in CT and MT plots, respectively. Then, a cultivator pass was carried out for both tillage systems. Thus, crop residues were almost completely incorporated into the soil in CT, whereas under MT they were covered over approximately 30% of the plot surface with the previous season's crop residues. No tillage involved direct drilling and spraying with glyphosate (at a rate of 2 L ha<sup>-1</sup> of Sting Monsanto ®) for weed control, and previous season's crop residues were retained on the soil surface. Different types of crop residues were applied to the soil in the rotation treatment, depending on rotation phase. Since wheat was preceded by fallow, the relatively little biomass generated during that phase was left or incorporated into the soil surface in the following crop, winter wheat. By contrast, in monoculture wheat, straw residue provided a greater N and C input (235 Mg C ha<sup>-1</sup>; 20 kg N ha<sup>-1</sup>) to the following crop of wheat. Rotational and monoculture wheat were sown on 26<sup>th</sup> November 2011 and 14<sup>th</sup> November 2012 in campaign 1 and 2, respectively, with 210 kg seed ha<sup>-1</sup>. Fertilizer was applied at seeding (16 kg N ha<sup>-1</sup> as NPK, 8-24-8) in both campaigns and at dressing as ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>, 27-0-0) on 22<sup>nd</sup> March 2011 and 11<sup>th</sup> March 2012. The N fertilization rate at dressing was calculated by taking into account the expected crop yield and soil mineral N content two weeks before fertilizer application (February). There was higher average nitrate (NO<sub>3</sub><sup>-</sup>-N) content in the 0-15 cm soil at dressing fertilization in campaign 1 (27



mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$ ) than in campaign 2 (5.6 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$ ), which resulted in different N rates in campaign 1 (11 kg N  $\text{ha}^{-1}$ ) and 2 (54 kg N  $\text{ha}^{-1}$ ). All treatments received post-emergency herbicide treatments (HerbimurDoble ®) at a rate of 1.6 L  $\text{ha}^{-1}$  for both campaigns. Wheat was harvested on 10<sup>th</sup> June 2012 and 18<sup>th</sup> June 2013, for campaign 1 and 2, respectively.

### 2.3. GHG emissions sampling and analyzing

Fluxes of  $\text{N}_2\text{O}$  and  $\text{CH}_4$  were measured from October 2011 to October 2013, using the static chamber technique (Sanz-Cobena et al., 2014). One chamber (diameter 35.6 cm, height 19.3 cm) was placed in each subplot and closed (for 1 h) by fitting them into stainless steel rings, which were inserted after plough events into the soil to a depth of 10 cm to minimize the lateral diffusion of gases and avoid the soil disturbance associated with the insertion of the chambers in the soil. They were only removed during management practices. Samples were always taken with wheat plants inside the chamber. Thermometers were placed inside three randomly selected chambers during the closure period of each measurement in order to correct the fluxes for temperature. When plants exceeded the chamber height (19.3 cm), plastic intersections of 19 cm were used between the ring and the chamber.

Gas samples were taken three times per week during the first and second week, then twice per week during the first month after fertilization events or during rainfall periods and then, every week or every two weeks until the end of the cropping period. After harvest, one gas sample was taken each month. To minimize any effects of diurnal variation in emissions, samples were taken at the same time of day (10–12 am).

Gas samples (20 mL) were taken at 0, 30 and 60 min to test the linearity of gas accumulation in each chamber. Samples were analyzed by gas chromatography using a

HP-6890 gas chromatograph equipped with a headspace autoanalyzer (HT3), both from Agilent Technologies (Barcelona, Spain). HP Plot-Q capillary columns transported gas samples to a  $^{63}\text{Ni}$  electron-capture detector (Micro-ECD) to analyze  $\text{N}_2\text{O}$  concentrations and to a flame ionization detector (FID) connected to a methanizer to measure  $\text{CH}_4$ . The temperatures of the injector, oven and detector were 50, 50 and 350°C, respectively.

The increases in GHG concentrations within the chamber headspace were generally linear ( $R^2 > 0.90$ ) during the sampling period (1h). Therefore, emission rates of fluxes were estimated as the slope of the linear regression between concentration and time (after corrections for temperature) and from the ratio between chamber volume and soil surface area (Abalos et al., 2014). Cumulative  $\text{N}_2\text{O}$ -N and  $\text{CH}_4$ -C emissions per subplot during the sampling period were estimated by linear interpolations between sampling dates, multiplying the mean flux of two successive determinations by the length of the period between sampling and adding that amount to the previous cumulative total (Sanz-Cobena et al., 2014).

## *2.5 Soil and crop analyses, meteorological data*

In November 2011, composite soil samples were collected from each subplot at depths of 0-7.5 cm, 7.5-15 cm and 15-30 cm. Soil samples were air-dried and sieved. Then, SOC was determined following the wet oxidation method (Nelson and Sommers, 1996). In addition, bulk density was determined using intact core samplers as described by Grossman and Reinsch (2002). Bulk density was measured once a year (before the start of the experiment, as indicated above), on the basis that although bulk density diminishes in the topsoil layer immediately after tillage, this effect is short-lived and is followed by a rapid reorganization of the soil (Gómez-Paccard et al. 2015). In order to relate gaseous emissions to soil properties, soil samples were collected from 0-15 cm

depths during the growing season on almost all gas-sampling occasions. Three soil cores (2.5 cm diameter and 15 cm length) were randomly sampled close to the ring in each subplot, and then mixed and homogenized in the laboratory. Dissolved organic C (DOC) was determined by extracting 8 g of homogeneously mixed soil with 50 mL of deionized water. Afterwards, DOC content was analyzed with a total organic carbon analyser (multi N/C 3100 Analytik Jena) with an IR detector. Soil ammonium ( $\text{NH}_4^+$  - N) and  $\text{NO}_3^-$  -N concentrations were analyzed using 8 g of homogeneously mixed soil extracted with 50 mL of KCl (1M), and measured by automated colorimetric determination using a flow injection analyzer (FIAS 400 Perkin Elmer) with a UV-V spectrophotometer detector. The water-filled pore space (WFPS) was calculated by dividing the volumetric water content by total soil porosity. Total soil porosity was calculated according to the relationship: soil porosity =  $(1 - \text{soil bulk density}/2.65)$ , assuming a particle density of  $2.65 \text{ g cm}^{-3}$  (Danielson et al., 1986). Gravimetric water content was determined by drying soil samples at  $105^\circ\text{C}$  in a MA30 Sartorius® oven.

Grain yield and above-ground biomass were measured by harvesting two randomly selected  $0.5 \times 0.5 \text{ m}$  squares from each subplot. Aerial biomass was cut by hand at the soil level and weighted after separating grain and straw. The total N content of grain and straw were determined with an elemental analyzer (TruMac CN Leco).

## *2.6. Yield-scaled $\text{N}_2\text{O}$ emissions, N surplus and GWP calculations*

Yield-scaled  $\text{N}_2\text{O}$  emissions, expressed as g  $\text{N}_2\text{O}$ -N per of kg N uptake, were calculated based on van Groenigen et al. (2010), considering total above-ground N uptake (wheat grain and straw). The N surplus was calculated as the above-ground N uptake of the crop minus the N fertilizer applied, in  $\text{kg N ha}^{-1}$  (van Groenigen et al., 2010). Carbon sequestration in the first 30 cm of soil and  $\text{CO}_2$  emissions from fuel used

in farm operations (e.g. tillage, herbicide and fertilizer application, seeding, harvest) and from manufacturing inputs (operation GHG emission + input GHG emission) were calculated as described by Guardia et al. (2016). The “ $\Delta$  soil C GWP” component, as an indicator of the soil C balance, was calculated taking the difference in SOC stocks between monoculture wheat-CT (as baseline) and the other treatments. To avoid the bias associated to bulk density, the comparison of C stocks was made on a fixed soil mass basis, as described in Ellert and Bettany (1995). Default values of GHG emissions derived from farm operations and manufacturing inputs have been reported by West and Marland (2002), Lal (2004) and Snyder et al. (2009).

## *2.7. Statistical analysis*

Statistical analyses were carried out with Statgraphics Plus 5.1. Analyses of variance (two-way ANOVA) were performed for almost all variables in the experiment for both campaigns (except climatic ones). A three-way ANOVA was also carried out in order to assess the effect of each campaign and the possible interactions among factors (campaign, tillage and crop). The normality and variance uniformity of data were assessed by the Shapiro-Wilk test and Levene’s statistic, respectively, and log-transformed before analysis when necessary. Means were separated by Tukey's honest significance test at  $P < 0.05$ . For non-normally distributed data (mean soil  $\text{NH}_4^+$  content and YSNE in campaign 1 in the three-way ANOVA), the Kruskal–Wallis test was used on non-transformed data to evaluate differences at  $P < 0.05$ . Linear regression analyses were carried out to determine relationships between cumulative gas fluxes and soil parameters, with a 95% significance level.

## **3. Results**

### *3.1. Environmental conditions, soil C and mineral N contents*

Total rainfall accounted for 193.6 mm and 369 mm, in campaign 1 (from October 2011 to June 2012) and campaign 2 (from October 2012 to June 2013), respectively (Fig. 1a). Campaign 1 was one of the most dry crop campaigns since 1994; the mean rainfall value from 1994-2013 period was 365.1 mm. Soil WFPS content (Fig. 1b) in the upper soil layer was dependent on rainfall events and tillage. For both crop campaigns, WFPS values of NT were often maintained above those of CT or MT. For NT the number of days with WFPS above 50% was 25-48 and 72-88 days in campaign 1 and 2, respectively; whereas those for CT were 10-15 and 25-35 days; and those for MT were 4-8 days in both campaigns.

Topsoil  $\text{NH}_4^+$  content (Fig. 2a, b) peaked after each fertilization event. However, The  $\text{NH}_4^+$  concentration decreased rapidly reaching background values ( $< 10 \text{ mg NH}_4^+ - \text{N kg}^{-1}$ ) after 10-35 days of basal and dressing fertilization. Average  $\text{NH}_4^+$  values did not show significant differences between tillage and cropping systems, but were significantly smaller ( $P < 0.05$ ) in campaign 1 than in campaign 2. The soil  $\text{NO}_3^-$  content in the topsoil (Fig. 2c, d) also increased after fertilization events in both campaigns and ranged between 0.80 and 59.1  $\text{mg NO}_3^- - \text{N kg}^{-1}$ . No differences between cropping systems (wheat in rotation versus continuous cropping of wheat) were observed, while soil mean  $\text{NO}_3^-$  content was greater ( $P < 0.05$ ) in NT plots than in the other tillage treatments in the campaign 1. In campaign 2, differences were not significant between tillage or cropping treatments. Despite lower N application rates, the average  $\text{NO}_3^-$  content was higher in campaign 1 than in campaign 2 ( $P < 0.05$ ).

The DOC content of the topsoil (0-15 cm) (Fig. 2e, f) ranged from 57.2 to 205.4  $\text{mg C kg}^{-1}$  (campaign 1) and from 29.4 to 170.2  $\text{mg C kg}^{-1}$  (campaign 2). The mean DOC content for NT (taking into account the whole crop period) was significantly higher than those for MT and CT (27 and 50% for campaign 1; 36 and 42% for

campaign 2, respectively). No significant differences were found between cropping systems and campaigns. The SOC content in the upper layer was significantly increased after 17 years of NT, as opposed to MT and CT (Table 1). The highest SOC concentrations in the 15-30 cm layer were observed in CT ( $P < 0.05$ ), but the SOC stock of the three soil layers (0-30 cm) was significantly higher in NT treatment. With regards to the cropping effect, monoculture wheat also tended to increase SOC sequestration compared with rotational wheat ( $0.05 < P < 0.10$ ).

### 3.2 $N_2O$ and $CH_4$ emissions

Nitrous oxide fluxes (Fig. 3) ranged from -0.18 to 0.46 mg  $N_2O$  -N m<sup>-2</sup> d<sup>-1</sup>. The highest emission peaks occurred after seeding and top-dressing fertilization in both campaigns (especially in campaign 2) and also after some rainfall events. Negative  $N_2O$  fluxes were measured on several occasions for all treatments during both campaigns. The data from both campaigns showed that  $N_2O$  emissions were not affected by tillage or crop (Table 2), but significant interactions of tillage and crop with the campaign factor were reported. In campaign 1, cumulative  $N_2O$  emissions (Table 2) were significantly lower for NT than those for MT and CT, while any significant crop effect or tillage\*crop interactions were found. With regards to campaign 2, higher cumulative  $N_2O$  emissions ( $P < 0.05$ ) were observed in wheat in monoculture (with respect to rotational wheat), without no significant effect of tillage or the interaction of factors. Total cumulative  $N_2O$  fluxes were greater ( $P < 0.05$ ) in campaign 2 than in campaign 1. The ratio of  $N_2O$  -N emitted per mineral N applied was significantly greater ( $P < 0.05$ ) during campaign 1 (0.52%) than during campaign 2 (0.28%) (data not shown).

Methane emissions ranged from -1.32 to 0.46 mg  $CH_4$  -C m<sup>-2</sup> d<sup>-1</sup> (data not shown). Therefore, all treatments were sinks for  $CH_4$  during almost all of the

experimental period, although positive fluxes were observed on some sampling events. In both campaigns, net CH<sub>4</sub> oxidation (Table 2) was significantly lower in the monoculture wheat than in rotational wheat, whereas no significant effect of tillage was reported ( $P > 0.05$ ). In campaign 1, a significant and negative correlation was found between CH<sub>4</sub> fluxes and NH<sub>4</sub><sup>+</sup>-N content ( $P < 0.05$ ,  $n = 20$ ,  $r = -0.52$ ). Methane emissions correlated with WFPS content in both campaigns ( $P < 0.05$ ,  $n = 20$ ,  $r = 0.50$ ).

### 3.3 Crop yield, YSNE and N surplus

Grain yield (Table 2) was significantly higher in campaign 2 than in 1 ( $P < 0.001$ ). Crop yield for both campaigns (three-way ANOVA), showed a significant interaction ( $P < 0.05$ ) between campaign and tillage: NT tended to increase (compared with CT) grain yield in the dry campaign (11/12) while the opposite tendency was observed in the normal campaign (12/13). On average, MT led to numerically (but not statistically) lower yields than NT and CT. Regarding cropping effect, grain yield in rotational wheat was significantly higher ( $P < 0.05$ ) than that in monoculture wheat.

In campaign 1, YSNE were significantly lower ( $P < 0.05$ ) for NT than those for MT and CT (Table 2), whereas no significant differences were observed for the crop effect. Considering data for both campaigns, MT and monoculture wheat significantly increased YSNE as opposed to NT and rotational wheat, respectively. However, a significant interaction of tillage with the campaign factor was observed, since NT and CT were the tillage treatments with most mitigated YSNE in campaign 1 and 2, respectively. The values for N surplus were significantly lower ( $P < 0.05$ ) in campaign 2 than campaign 1 (Table 2). There were no significant differences in N surplus values for the other effects (tillage, crop and interactions).

### 3.4 Global Warming Potential

The net GWP was significantly lower in NT, than MT and CT (Table 3). Wheat in monoculture tended to decrease the net GWP as a result of higher C sequestration, but differences were not statistically significant at 95% significance level. The GHG-GWP (soil N<sub>2</sub>O and CH<sub>4</sub> fluxes) component was significantly affected by tillage and crop factors, since CT and monoculture wheat significantly increased CO<sub>2</sub>-eq emissions compared with NT and rotational wheat, respectively. The GWP was higher during campaign 2 as a result of higher N fertilizer input (Fig. 4). Wheat in rotation only resulted in higher C sequestration than the conventional monoculture wheat-CT management under NT.

## **4. Discussion**

### *4.1 Effect of campaign, tillage and crop systems on N<sub>2</sub>O emissions*

The main factor affecting N<sub>2</sub>O emissions in this experiment was N input (from chemical fertilizer and crop residues), which was very dependent on the campaign and the soil moisture, which in turn was influenced by rainfall amount and distribution. In this context, N<sub>2</sub>O fluxes were significantly higher in campaign 2 (with the highest N input and rainfall) (Table 2). Due to the complexity of factors and processes affecting the release of N<sub>2</sub>O emissions, the effect of tillage and crop factors was not consistent throughout both campaigns, so that the interactions need to be analyzed in detail. Contrary to our hypothesis, tillage systems did not have any significant effect on N<sub>2</sub>O emissions when the data from both campaigns are considered (Table 2). Our results were in agreement with those of Tellez-Rio et al. (2015) and Guardia et al. (2016) under similar climatic conditions. As observed for tillage, the crop effect did not influence N<sub>2</sub>O emissions across the 2 campaigns. These results could be explained by the similar rates of synthetic N which was applied to both cropping systems, although a significant



interaction (Table 2) with the campaign effect (i.e. higher N<sub>2</sub>O losses in monoculture wheat than in rotational wheat, but only in the second campaign) was observed. This interaction suggests that the effect of residues from previous crops can be comparable and even higher than that of synthetic fertilizers (Lenka and Lal, 2013), especially in calcareous soils and low-input semi-arid cropping systems. Additionally, the effect of tillage was not consistent in the two campaigns, since NT significantly reduced N<sub>2</sub>O losses during campaign 1 but not during campaign 2 (normal precipitation and N input) compared with to MT or CT. That caused the tillage\*campaign interaction to be significant at 10% significance level.

The meta-analysis of van Kessel et al. (2013) reported a significant mitigation of N<sub>2</sub>O emissions under NT in dry climates and long-term (> 10 years) studies. Lower emissions following long-term adoption of NT were explained as a result of the improvements of SOC content and porosity, thus reducing the formation of anaerobic microsites (Six et al., 2004). Lower emissions were generally observed under NT in our study for both campaigns in the rotational wheat system and also for monoculture wheat in campaign 1, supporting the results of Van Kessel et al. (2013). Conversely, the results of monoculture wheat in campaign 2 did not agree with this study, because the monoculture wheat-NT treatment resulted in relatively high N<sub>2</sub>O fluxes during this campaign, particularly after dressing fertilization (Fig. 5). Therefore, we hypothesized that the influence of the climatic conditions (particularly rainfall) and tillage (incorporating/leaving the residue on surface) on the mineralization of previous crop residues (whose amount was different between cropping systems, as explained in section 2.2) drove the N<sub>2</sub>O emission pattern in our experiment. In the case of rotational wheat, an important part of crop residue was presumably mineralized during fallow period (the previous year of rotational wheat growing phase), so N<sub>2</sub>O fluxes may have

been less dependent on the interaction of crop residue and mineral fertilizer than in continuous cropping of the winter wheat. However, in campaign 1, differences in N<sub>2</sub>O emissions due to crop residue inputs were not observed between cropping systems. We hypothesized that the low rainfall amounts in campaign 1 limited soil water availability, particularly soil moisture content, which was not enough to promote an intensive N mineralization and crop residues turnover, hence not stimulating N<sub>2</sub>O production (Mutegi et al., 2010). The number of days with a WFPS above 50%, which has been suggested as a threshold for highest N<sub>2</sub>O losses (Linn and Doran, 1984; Li et al., 2016) was lower in campaign 1 (from 4 to 48 days) than in campaign 2 (from 7 to 88 days), depending on tillage system, supporting our findings.

By contrast, the N<sub>2</sub>O emissions during campaign 2 were higher in monoculture wheat than in rotational wheat. This effect could be a result of better environmental conditions for the mineralization of crop residues from the previous year (Chen et al., 2013). In monoculture wheat, a combination of residue inputs with a high C:N ratio (mean C:N ratio of 160.3) and mineral N fertilizer, both at seeding and dressing, may have stimulated denitrification losses from mineral N added to soil (Li et al., 2016), as residues provide an energy supply for denitrifying microorganisms (Sarkodie-Addo et al., 2003; Sanz-Cobena et al., 2014). This effect was particularly noticeable after dressing fertilization in the campaign 2, increasing fluxes in the monoculture wheat-NT treatment and changing the trend observed in the first campaign and the beginning of the second (Fig. 5). We hypothesized that the slower mineralization of non-incorporated wheat residues in NT (with respect to MT/CT) favored the N<sub>2</sub>O release from the interaction of dressing synthetic N and the mineralization of wheat residues, during the stage (spring) and the campaign (2, as opposed to the dry campaign 1) with more favorable conditions for mineralization (Abalos et al., 2013; Guardia et al., 2016).

## 4.2 CH<sub>4</sub> emissions

In this long-term tillage study, cumulative emissions provided a net CH<sub>4</sub> sink in all tillage and cropping systems (Table 2), as generally reported in agricultural soils under semiarid conditions (Snyder et al., 2009). The negative correlation found between soil NH<sub>4</sub><sup>+</sup> content and CH<sub>4</sub> fluxes ( $P < 0.05$ ) in campaign 1 did not agree with previous studies (e.g. Hütsch et al., 1996), which suggested a competitive inhibition of the enzyme responsible for the oxidation of CH<sub>4</sub> (CH<sub>4</sub> monooxygenase) with the NH<sub>3</sub> monooxygenase (Le Mer and Roger, 2001). Conversely, the meta-analysis of Aronson and Helliker (2010) reported that low amounts of N (<100 kg ha<sup>-1</sup>) tend to stimulate methanotrophy, while larger rates are inhibitory. This explains the correlation obtained in our study, in which low N rates were used, particularly during campaign 1.

Tillage systems did not produce significant differences in CH<sub>4</sub> uptake in any campaign, which is consistent with results reported by Guardia et al. (2016) and Tellez-Rio et al. (2015), under semiarid Mediterranean conditions. However, some authors have suggested that the improvement of soil structure in NT, associated with increases in macroporosity and reduction of anaerobic microsites, can favor CH<sub>4</sub> consumption (Plaza-Bonilla et al., 2014). Our results may have been a consequence of similar topsoil porosity in all tillage systems and the low soil moisture content maintained during campaign 1 and 2.

Greater CH<sub>4</sub> oxidation ( $P < 0.05$ ) was found in rotational wheat than in monoculture wheat in both campaigns, which would suggest that soil conditions under this rainfed rotation can be more favorable for methanotrophic microorganisms. The incorporation of high C:N crop residues has been reported to increase CH<sub>4</sub> emissions (Le Mer and Roger, 2001), and that may have partially offset the CH<sub>4</sub> oxidation in

monoculture wheat subplots, where a higher amount of straw was retained/incorporated. This was also reported by Lenka and Lal (2013), who showed that CH<sub>4</sub> uptake capacity was increased in bare soil when compared to treatments with residue amendment.

#### *4.3 Grain yield, YSNE and N surplus*

Grain yield was affected by campaigns, which decreased almost 50% in the dry campaign 1 compared to campaign 2, due to the low rainfalls measured in campaign 1. The tillage\*campaign interaction in wheat yields showed that the most productive tillage system was dependent on climate and management conditions: NT increased grain yield compared to MT / CT in campaign 1 whereas CT produced higher yield than NT / MT in campaign 2, although the differences were not statistically significant at 95% probability level. Controversy still exists about crop yield declines in NT, but CT overall leads to higher crop yields in experiments with high water and nutrient availability (Chatskikh and Olesen, 2007), whereas in semiarid agroecosystems, increases in water content and soil fertility achieved with NT adoption can result in higher yields (Morell et al., 2011; Plaza-Bonilla et al., 2014). Recently, the meta-analysis of van Kessel et al. (2013) reported that long-term NT in dry climates had no significant effect on yield compared to CT, but NT generally produced a yield decline. Although, differences in yield between tillage systems were not observed in this experiment in any campaign, our results seem to suggest that NT enhanced yield with limited rainfall values below 200 mm (campaign 1), whereas higher rainfall ( > 300 mm) increased yield in CT (campaign 2). Our results were consistent with De Vita et al. (2007) under Mediterranean conditions who explained the superior effect of NT relative to CT due to lower water evaporation from soil combined with enhanced soil water availability. Considering the average 2-campaigns data, MT resulted in numerically but not statistically lower yield than those of CT or NT. The increased weed pressure in this

tillage system (Armengot et al., 2015) was also observed in our experimental site, and could explain this tendency. With regard to crop effect, monoculture wheat significantly reduced grain yield compared with rotational wheat, especially in campaign 2. Our results confirm the positive effect of crop rotation on wheat yield under semi-arid conditions (López-Bellido and López-Bellido, 2001).

The YSNE from our study were in the lowest range of values reported by van Groenigen et al. (2010). These results indicate that rainfed semi-arid agro-ecosystems with adjusted N rates result in low N<sub>2</sub>O emissions per kg of N uptake. Since grain yield was not high (compared with other wheat cropping areas), these low YSNE were a result of small N<sub>2</sub>O losses, ranging from 0.07 to 0.23 kg N<sub>2</sub>O ha<sup>-1</sup> yr<sup>-1</sup>, compared to those (0.04-21.21 kg N<sub>2</sub>O ha<sup>-1</sup>) for European arable sites (Rees et al., 2013). Besides the small N<sub>2</sub>O emissions due to the low N fertilization rates, the low ratio of N<sub>2</sub>O -N emitted per mineral N applied (see section 3.2) confirms that the N<sub>2</sub>O emission factors of rainfed semi-arid areas are much lower than the IPCC default value (Aguilera et al., 2013b; Cayuela et al., 2016). In this type of agro-ecosystem, N<sub>2</sub>O emissions during winter are substantially limited by soil temperatures, while low WFPS is the main limiting factor for large N<sub>2</sub>O losses during spring (when most growth of winter crops occurs). Additionally, low SOC contents (Ussiri and Lal, 2012) and high soil pH conditions (Baggs et al., 2010), as was the case for our experimental site, may have also contributed to low N<sub>2</sub>O losses and YSNE. As a consequence, the mean values of N surplus (Table 2) were below the threshold (20-50 kg N ha<sup>-1</sup>) of an exponential increase of YSNE (van Groenigen et al., 2010). Remarkably, N surplus was significantly higher in the first campaign, with the driest conditions, in spite of the lower rate of application of synthetic N. This would suggest that there was inefficient uptake of N uptake under

water stress conditions, resulting in very low grain yields, without higher N<sub>2</sub>O losses due to unfavorable soil WFPS, as explained above.

Our results highlight the importance of crop rotation as an effective YSNE mitigation strategy, due to increased yields and similar (or lower) N<sub>2</sub>O losses as continuous cropping of wheat. The tillage\*campaign interaction for grain yields and the low N<sub>2</sub>O fluxes drove the tillage\*campaign interaction observed for YSNE. Overall, NT significantly mitigated YSNE as opposed to MT, which had no effect on area-scaled N<sub>2</sub>O losses but was a less advantageous tillage management considering the YSNE ratio. In the campaign with less rainfall than the average, NT mitigated YSNE, as observed by van Kessel et al. (2013) for long-term studies under dry conditions, so it emerges as an interesting option in a global change context with increased aridity. In contrast, in normal rainfall campaigns CT arises as the most sustainable alternative for increasing grain yields while leading to similar N<sub>2</sub>O losses as NT.

#### *4.4 Global Warming Potential*

Almost all treatments (except rotational wheat-NT) had positive GHG-GWP emission values (19-204 kg CO<sub>2</sub>-eq ha<sup>-1</sup>), showing that in spite of low N<sub>2</sub>O fluxes, CH<sub>4</sub> oxidation did not offset N<sub>2</sub>O losses (Fig. 4). As reported by previous studies (e.g. Aguilera et al., 2013a; 2015; Plaza-Bonilla et al., 2015; Abdalla et al., 2016), NT significantly increased C sequestration compared with CT (Table 3). This occurred despite the higher SOC content in the 15-30 cm layer in CT (as opposed to NT/MT), as suggested by Baker et al. (2007). Carbon sequestration was the main cause of the differences between tillage and crop treatments (Fig. 4), but CO<sub>2</sub>-eq emissions from inputs and operations were also important, a finding which is consistent with Aguilera et al. (2015) or Guardia et al. (2016). Therefore, our results indicate that management

practices which promote an increase in C stocks (e.g. NT) should be recommended in semi-arid areas. Supporting our findings, the recent meta-analysis of Abdalla et al. (2016) pointed out that the abatement of CO<sub>2</sub>-eq emissions through NT adoption is significantly higher in arid climates with low SOC content, as opposed to CT. Nitrous oxide (N<sub>2</sub>O) emissions have shown to carry less weight in GWP estimates than in previous studies (Mosier et al., 2006; Adviento-Borbe et al., 2007), but uncertainties associated with C sequestration dynamics and its calculation (Guardia et al., 2016) and the large climatic variability in rainfed semi-arid cropping areas, suggest that strategies that mitigate CO<sub>2</sub>-eq from other GWP components (N<sub>2</sub>O losses and inputs, e.g. by adjusting N rates) must be also considered.

Regarding crop effect, wheat in rotation tended to decrease C sequestration and consequently to enhance GWP ( $0.05 < P < 0.10$ ). Although the wheat phase of the rotation led to numerically higher CO<sub>2</sub>-eq than monoculture wheat, the widespread fallow-cereal-legume-cereal rotations provide further opportunities to mitigate the GWP during the legume and fallow phases, when lower (or zero) fertilizer inputs are applied.

## **5. Conclusions**

Our results showed that cumulative N<sub>2</sub>O emissions and YSNE were low in this long-term experiment carried out under rainfed semiarid conditions with adjusted N inputs. On average, no significant effect of tillage or cropping system (wheat in rotation and in monoculture) was observed. But this simple overview hides a more complex underlying story; N<sub>2</sub>O emissions were increased in a normal campaign in monoculture wheat (due to the mineralization of previous wheat residues), as opposed to rotational wheat, and decreased in a dry campaign in NT, as opposed to MT/CT. Therefore, Conservation Agricultural practices (NT and rotation) resulted in similar or lower N<sub>2</sub>O

losses than conventional ones. Methane uptake was significantly higher in rotational wheat than in monoculture wheat, while no effect of tillage was observed. Grain yield and consequently YSNE were strongly affected by climatic variability, since NT and CT resulted in significantly higher productivities and lower YSNE in the dry and the normal campaigns, respectively. Wheat in rotation significantly mitigated YSNE, as opposed to monoculture wheat. Higher C sequestration caused NT to reduce Net GWP compared with the rest of tillage treatments. No-till should be recommended in semi-arid areas to mitigate the Net GWP of semi-arid agro-ecosystems, providing the opportunity to reduce YSNE in dry years and therefore in a global change scenario. By contrast, MT performed less well on the basis of YSNE and GWP balances. Wheat in rotation tended to increase Net GWP, but the abatement of YSNE and the opportunities for reducing input CO<sub>2</sub> emissions during other rotation phases (fallow and/or legume) may provide and optimum balance between grain yields and GHG mitigation.

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## Figure captions

**Fig. 1a** Weekly mean soil temperature (°C) and rainfall (mm) and **b** evolution of soil WFPS (%) in the different tillage (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping (rotational wheat, W, and monoculture wheat, M) treatments during both crop campaigns.

**Fig. 2a, b** NH<sub>4</sub><sup>+</sup>-N; **c, d** NO<sub>3</sub><sup>-</sup>-N; and **e, f** DOC concentrations in the 0–10 cm soil layer during both crop campaigns for the different tillage (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) treatments. Data are provided separately for rotational wheat (W, right) and monoculture wheat (M, left) treatments. The arrows indicate the dates of application of synthetic N. Vertical lines indicate standard errors.

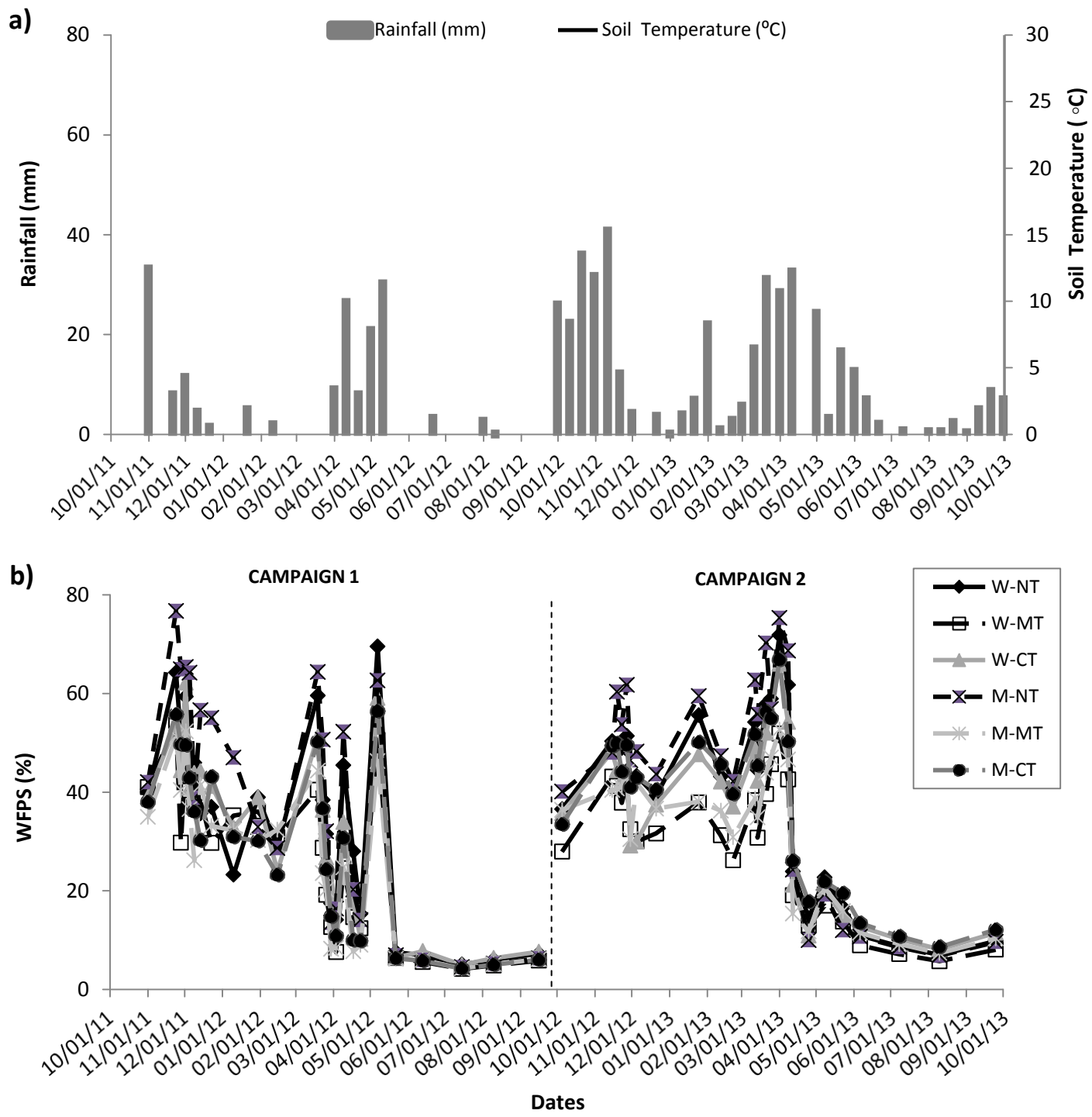
**Fig. 3** Fluxes of N<sub>2</sub>O-N during both crop campaigns for the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping systems: **a** rotational wheat (W), and **b** monoculture wheat (M). The arrows indicate the dates of application of synthetic N. Vertical lines indicate standard errors.

**Fig. 4** Relative contribution of each component to Net Global Warming Potential (GWP) in each tillage (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping treatment (rotational wheat, W, and monoculture wheat, M) during both crop campaigns.



**Fig. 5** Cumulative N<sub>2</sub>O-N emissions during both crop campaigns for the different tillage (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping (rotational wheat, W, and monoculture wheat, M) treatments, from the beginning of the campaign to dressing fertilization (1<sup>st</sup> fertilization) and from dressing fertilization to the end of the campaign (2<sup>nd</sup> fertilization). Vertical lines indicate standard errors.

**Figure**  
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Figure

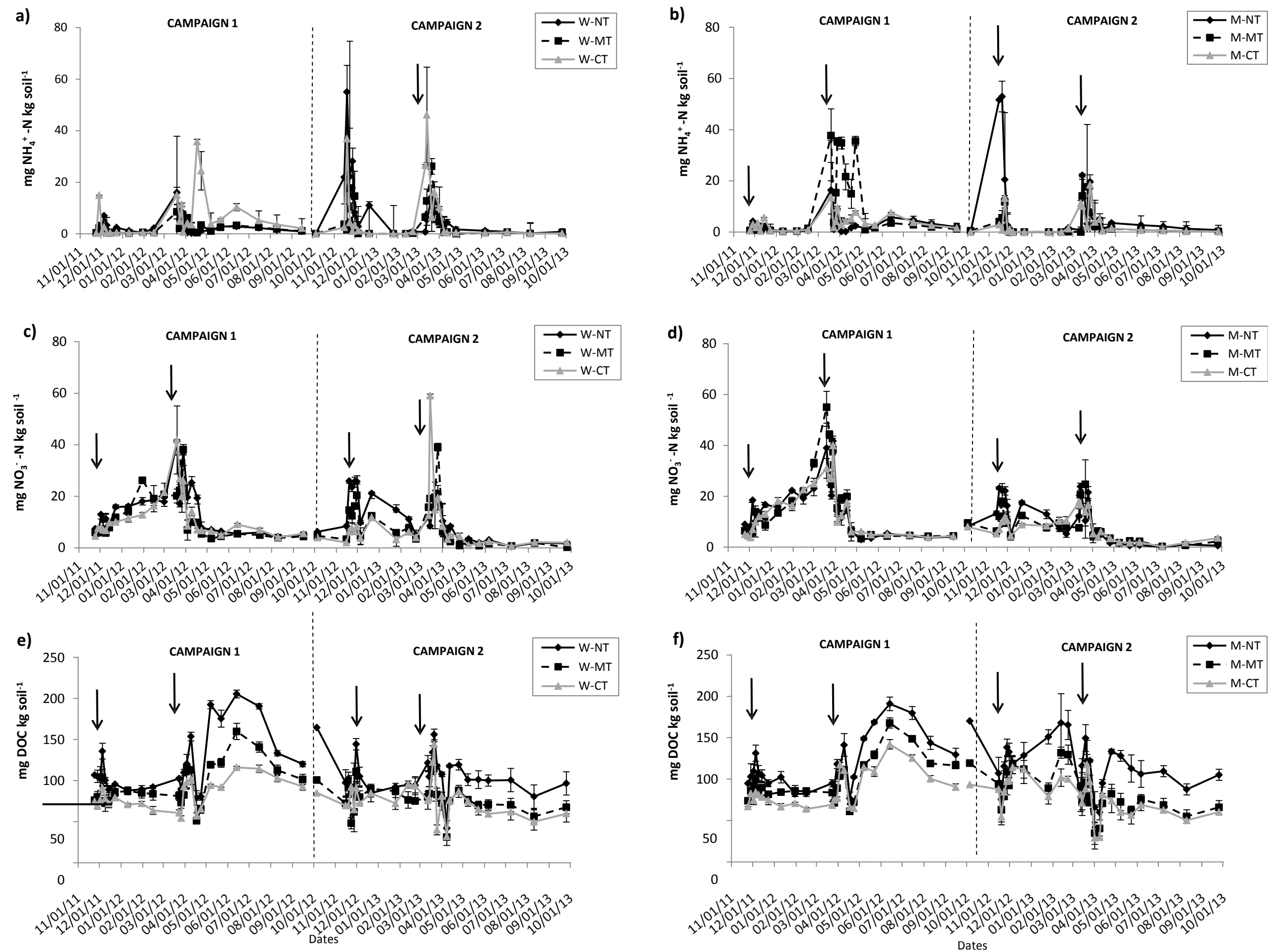
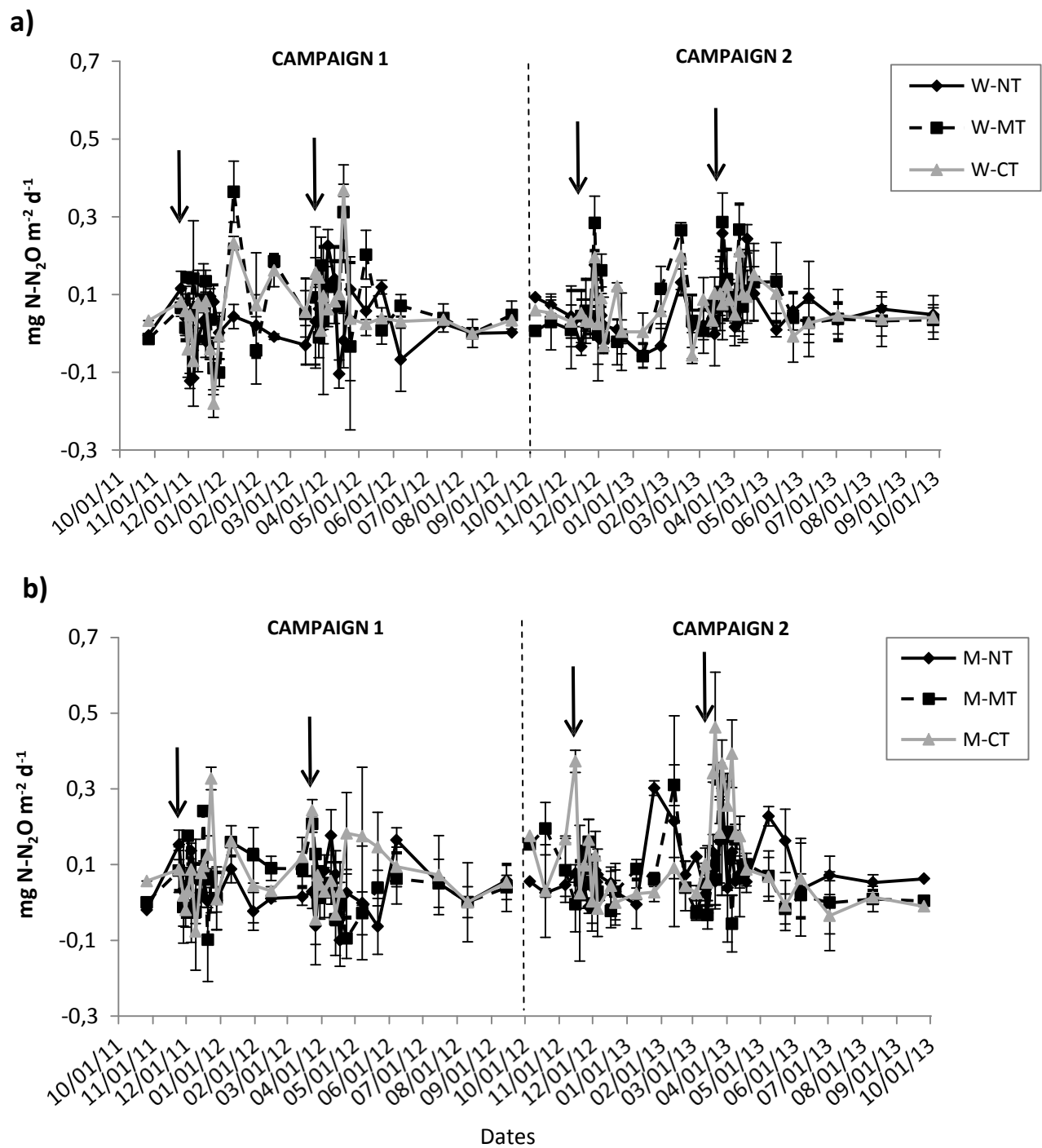
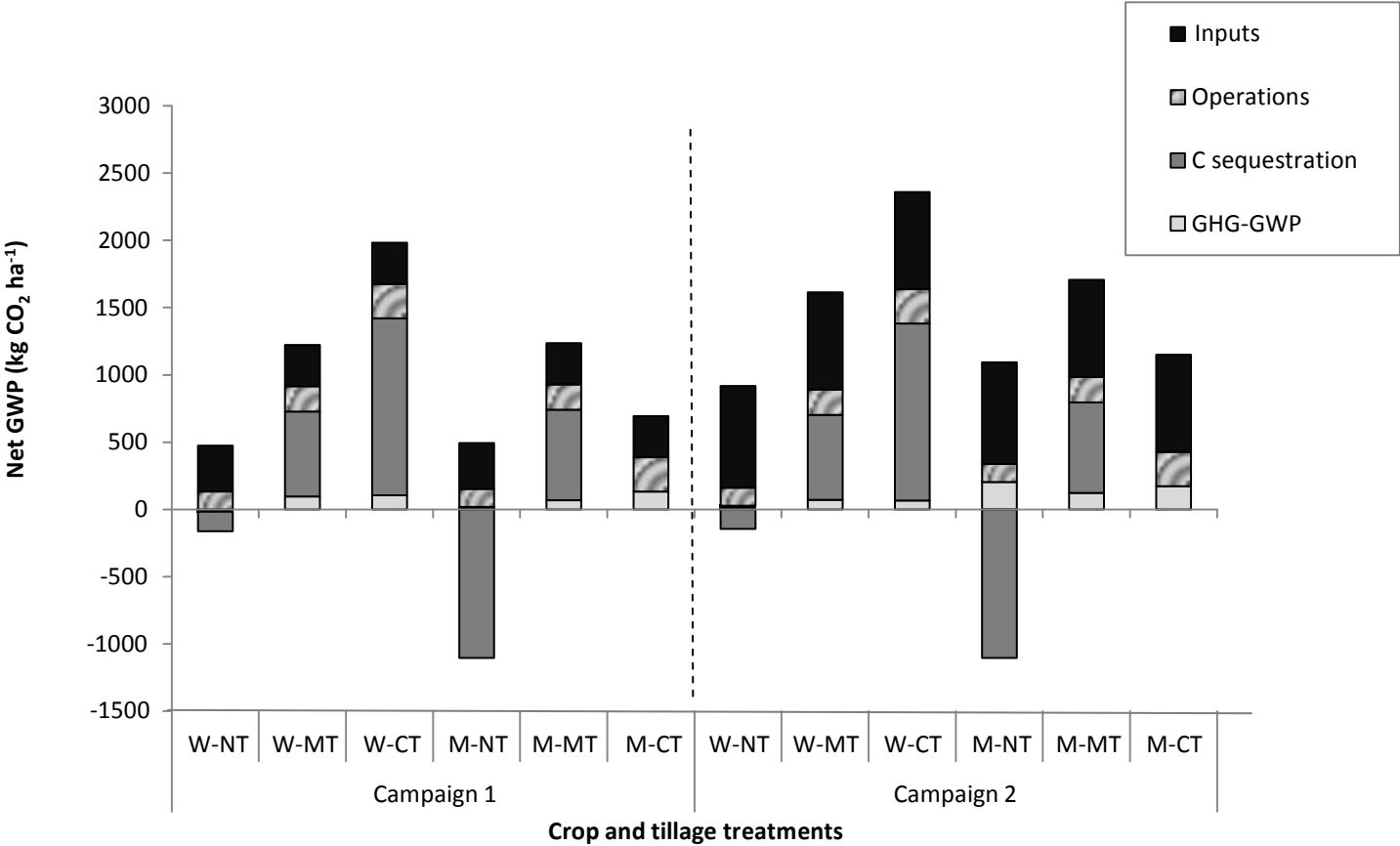
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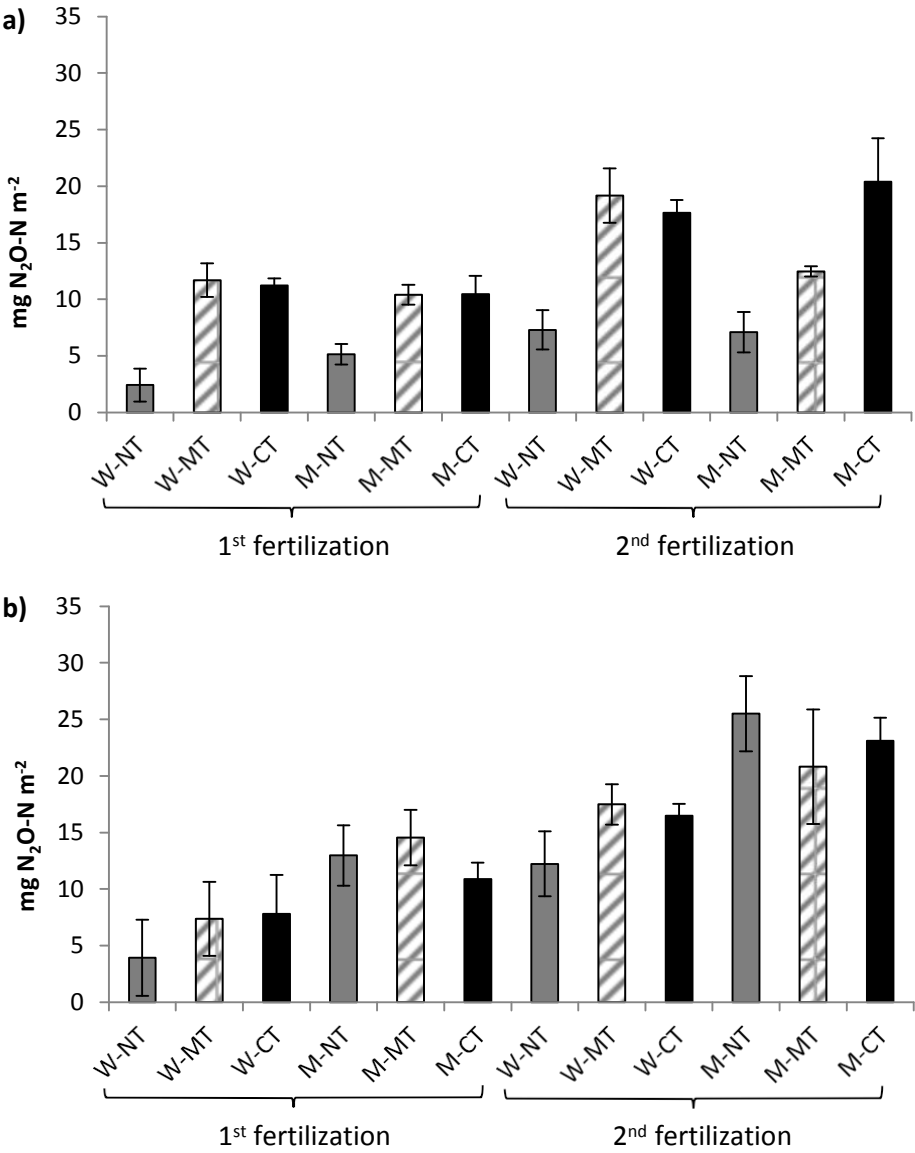


Figure

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**Figure**  
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**Table 1.**  
SOC content (g C kg<sup>-1</sup>) in the 0-7.5, 7.5-15 and 15-30 cm soil layers, total SOC content (Mg C ha<sup>-1</sup>) in the 0-30 cm depth of the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping system (rotational wheat, W, and monoculture wheat ,M).

Depth (cm)	SOC (g kg <sup>-1</sup> )			Total SOC (Mg ha <sup>-1</sup> )
	0-7.5	7.5-15	15-30	0-30
Tillage	<i>P</i> = 0.001	<i>P</i> = 0.516	<i>P</i> = 0.035	<i>P</i> = 0.033
NT	11.2 b	5.8	4.6 a	28.8 b
MT	6.7 a	5.4	4.6 a	22.9 a
CT	5.7 a	5.4	5.3 b	22.9 a
S.E.	0.7	0.3	0.2	1.4
Crop	<i>P</i> = 0.186	<i>P</i> = 0.008	<i>P</i> = 0.117	<i>P</i> = 0.070
W	7.2	4.9 a	4.7	23.1
M	8.5	6.1 b	5.1	26.6
S.E.	0.6	0.2	0.1	1.1
Tillage x crop	<i>P</i> = 0.713	<i>P</i> = 0.130	<i>P</i> = 0.098	<i>P</i> = 0.308

Different letters within columns indicate significant differences by applying the Tukey's honest significance test at *P* < 0.05. Standard Error (S.E.) is given for each effect.

**Table 2.**  
Total cumulative N<sub>2</sub>O-N and CH<sub>4</sub>-C fluxes, grain-yield, Yield-scaled N<sub>2</sub>O emissions (YSNE) and N surplus in the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping systems (rotational wheat, W, and monoculture wheat, M), in campaign 1 and campaign 2, and during the two seasons of the experiment.

Effect	N <sub>2</sub> O cumulative emission (g N <sub>2</sub> O-N ha <sup>-1</sup> yr <sup>-1</sup> )			CH <sub>4</sub> cumulative emission (g CH <sub>4</sub> -C ha <sup>-1</sup> yr <sup>-1</sup> )			Grain yield (kg grain ha <sup>-1</sup> )			YSNE (g N <sub>2</sub> O-N kg N up <sup>-1</sup> )			N surplus (Kg N ha <sup>-1</sup> )		
	Camp. 1	Camp. 2	2-season	Camp. 1	Camp. 2	2-season	Camp. 1	Camp. 2	2-season	Camp. 1	Camp. 2	2-season	Camp. 1	Camp. 2	2-season
Tillage	*	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*	ns	ns	ns
NT	72.0 a	181.6	130.3	-670.6	-654.2	-662.4	2068	3233	2650	1.7 a	3.4 b	2.5 a	21.6	-6.5	7.5
MT	158.1 b	191.5	174.9	-694.8	-866	-780.7	1151	3241	2196	5.3 b	3.1 ab	4.1 b	6.4	-4.4	1.0
CT	190.1 b	198.0	194.0	-639.6	-708.5	-674.0	1530	3885	2708	5.4 b	2.5 a	3.9 ab	12.0	13.2	12.6
S.E.	14.7	17.5	13.6	69.1	42.7	48.7	192	394	209	0.6	0.1	0.3	3.3	6.6	3.8
Crop	ns	*	ns	*	*	***	ns	ns	*	ns	**	*	ns	ns	ns
W	147.1	154.0 b	150.6	-792.0 a	-919.9 a	-855.9 a	1832.5	3903.2	2868 b	3.7	2.1 a	2.9 a	21.4	8.7	15.0
M	133.1	231.0 a	182.2	-544.6 b	-566.2 b	-555.4 b	1333.6	3003.3	2169 a	4.5	3.8 b	4.2 b	5.3	-7.2	-0.9
S.E.	28.0	18.0	12.5	62.6	82.7	29.7	240	322	171	0.9	0.3	0.4	4.9	7.2	4.0
Camp.			*			ns			***			*			*
1			140.1 a			-668.4			1583 a			4,1 b			13.3 b
2			192.7 b			-743.1			3453 b			3.0 a			0.8 a
S.E.			11.1			39.8			92			0.3			4.1
Till. x Crop	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Till. x Camp.			ns			ns			*			*			ns
Crop x Camp.			*			ns			ns			ns			ns



Different letters within columns indicate significant differences by applying the Tukey's honest significance test at\*  $P < 0.05$ , \*\* $P < 0.01$ , \*\*\*  $P < 0.001$ . "ns" means no significant. Standard Error (S.E.) is given for each effect.

**Table 3.**  
Estimated Global Warming Potential (GWP, kg CO<sub>2</sub> eq ha<sup>-1</sup> yr<sup>-1</sup>) for the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping systems (rotational wheat, W, and monoculture wheat, M).

Effect	Global Warming Potential (GWP, kg CO <sub>2</sub> eq ha <sup>-1</sup> yr <sup>-1</sup> )		
	GHG-GWP <sup>a</sup>	C sequestration <sup>b</sup>	Net GWP <sup>c</sup>
Tillage	<i>P</i> = 0.077	<i>P</i> = 0.008	<i>P</i> = 0.010
NT	58.6 a	-624.7 a	116.0 a
MT	89.8 ab	652.0 b	1444.8 b
CT	119.4 b	658.2 b	1546.7 b
S.E.	17.0	310.0	303.0
Crop	<i>P</i> = 0.009	<i>P</i> = 0.060	<i>P</i> = 0.078
W	58.4 a	600.8	1377.3
M	120.1 b	-143.8	694.4
S.E.	13.9	253.1	247.4
Campaign	<i>P</i> = 0.047	-	<i>P</i> = 0.189
1	67.5 a	-	806.1
2	111.0 b	-	1265.3
S.E.	13.9	-	247.4
Tillage x crop	<i>P</i> = 0.181	<i>P</i> = 0.332	<i>P</i> = 0.332
Tillage x campaign	<i>P</i> = 0.069	-	<i>P</i> = 0.988
Crop x campaign	<i>P</i> = 0.026	-	<i>P</i> = 0.883

Different letters within columns indicate significant differences by applying the Tukey's honest significance test at *P* < 0.05. Standard Error (S.E.) is given for each effect.

<sup>a</sup> Sum of CO<sub>2</sub> equivalents from N<sub>2</sub>O and CH<sub>4</sub> emissions, considering a 100-year horizon.  
<sup>b</sup> CO<sub>2</sub> equivalents from C sequestration, calculated taking the difference in SOC stocks between CT-M (as baseline) and the rest of tillage treatments, dividing it by the number of years since the experiment started (17) and considering the CO<sub>2</sub>/C molar ratio.

c Sum of CO<sub>2</sub> equivalents from N<sub>2</sub>O and CR. emissions, C sequestration, operations and inputs.